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Full Length Research

Phylodiversity of bla_{TEM} and bla_{CTX-M} genes of coliform isolates from ruminant mastitis in Plateau State Nigeria

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ABSTRACT: The study was conducted to determine the genetic characteristics, antibiotic susceptibility, and genetic relatedness of bla_{TEM}-type and bla_{CTX-M}-type ESBLs producing coliforms from ruminants suffering mastitis. In a cross sectional study, a total of 1052 milk samples were collected aseptically from ruminants across Plateau State, Nigeria. Bacterial culture and biotyping were performed according to standard guidelines. Phenotypic assay for ESBL production was carried out using the Brilliance ESBL Chromogenic Culture Medium (Oxoid, UK). Conventional PCR was used for amplification and detection of *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$ genes as described previously. Sequencing reactions were also performed in the Master Cycler pro 384 (Eppendorf) using the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the protocols supplied by the manufacturer. Sequences obtained were aligned with sequences deposited in the GenBank using the Basic Local Alignment Search Tool (BLAST) and phylogentic analysis performed MEGA software version 10.1.8. Coliforms isolated from milk samples included *Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogenes,* and *Serratia marcescens.* 677 coliforms were isolated from the 1052 milk samples across Plateau State, where *Escherichia coli* had the highest prevalence of 44% while *Serratia marcescens* had the least with 1.8%. Plateau South had the highest prevalence of 82.3%, followed by Plateau North and Plateau Central with 72.9% and 20.5% prevalence respectively. *E. aerogenes* recorded the highest resistance (85.71%) against Gentamycin, followed by *Serratia marcescens* with an 81.82% resistance against Gentamycin. The bla_{CTX-M} was more frequently isolated than bla_{TEM} having 24.39 and 12.19% prevalence respectively. The high-level of phylodiversity observed among ruminants means that there is transfer coliform harboring the bla_{CTX-M} and bla_{TEM} genes which might have been transmitted from clones of varying origins. The relatedness of the genes among organisms in different animals from diverse geographical locations demand a quick intervention that would reduce mortality and morbidity of animals and zoonotic transmissions. Sustained hygienic standards of livestock housing, milking procedures and processes, culling of chronically affected livestock, regular laboratory screening of pastoralists for infections can serve as good interventions.

Keywords: Antibiogram, β-lactamases, California Mastitis Test, ESBL, phylogeny, zoonosis.

INTRODUCTION

The use of antibiotics over the years have provided treatment and prevention for diseases, and served as growth promoters in livestock such as cattle, sheep, goat, swine and poultry (Sawant et al., 2005; CDC, 2007) thereby promoting and enhancing animal production and health (Abdellah et al., 2009). It is well known that any use of antimicrobials however appropriate and justified, contributes to the development of resistance and their widespread and unscrupulous misuse makes the situation worse. The frequent use of the most commonly used class of antibiotics in human medicine such as penicillins and the β-lactams (cephalosporins) has resulted in the emergence of antibiotics resistant bacteria.

In 1991, Ghuysen recorded that most gram-negative

bacteria possess naturally occurring chromosomally mediated β-lactamases which is brought about by selective pressure exerted by β-lactam producing soil organisms found in the environment (Ghuysen, 1991). These β-lactamases provide extended resistance to β-lactam antibiotics and are usually produced by a variety of coliforms such as *Escherichia coli*, *Klebsiella pneumoniae, Citrobacter* sp, and *Enterobacter* sp (Falagas and Karageorgopoulos, 2009; Tekiner and Ozpinar, 2016). They are commonly called Extended-spectrum β-lactamase (ESBL) bacteria. Among the 'priority pathogens', ESBL-producing *Enterobacteriaceae* have been identified as an emerging global health threat because of their increasing prevalence in livestock in recent years after being mostly isolated in humans (Enoch et al., 2012; Reuland et al., 2013).

Since the beginning of the millennium, *E. coli* and other *Enterobacteriaceae* species producing CTX-M-type extended-spectrum-lactamases have been more frequently isolated from humans, their environment and their companion animals (Harada et al., 2012), from food-producing animals (Overdevest et al., 2011; Dahmen, 2013), and retail meats, including chicken, beef, and pork (Overdevest et al., 2011), globally. The first β-lactamase discovered was in 1965 in *E. coli* isolated from a patient called Temoniera in Greece and as result the resistance gene was named TEM (Datta and Kontomichalou, 1965). TEM is the next most frequently isolated ESBL genes after CTX-M but often isolated with CTX-M genes rather than alone (Ali et al., 2016).

Mastitis is the most common disease of ruminants especially cows (Contreras and Rodríguez, 2011). Coliforms like *E. coli* and *K. pneumoniae* have been incriminated as life-threatening cause of clinical mastitis (Dahmen, 2013). Mbuk et al. (2016) isolated *Enterobacter, Citrobacter, Klebsiella, Serratia,* and *Proteus* from cows suffering mastitis. Geser et al. (2012) recorded that food-animals are reservoirs of ESBL-producing *E. coli,* and are zoonotic having the ability to be transferred from animals to man and vice versa through varying direct and indirect means. This was confirmed by Madec et al. (2012) who reported that there was a similarity between CTX-M-15 genes isolated from cows and those isolated from humans in their study. Thus, the objective of this work was to determine the genetic characteristics, antibiotic susceptibility, and genetic relatedness of bla_{TEM}-type and bla_{CTX-M}-type ESBLs producing coliforms from ruminants suffering mastitis.

MATERIALS AND METHODS

Sampling

A cross-sectional survey was carried out on lactating 160 cows, 103 ewes and 103 does in two Local Government Areas of each of the three Senatorial Zone across Plateau State, Nigeria. The lactating ruminants and quarter examination were based on clinical manifestation and California Mastitis Test (CMT) for subclinical mastitis. The first stream of milk (foremilk) was discarded before volumes of about 10 ml of all quarters or halves were aseptically collected into labelled sterile universal bottles (Zeryehun and Abera, 2017). The samples were kept at 4°C in a cooler and transported to the Microbiology Laboratory of Federal College of Animal Health and Production Technology Vom, Plateau State, Nigeria.

Culture and biotyping

Bacteriological examination was done following the procedures as described by Geser et al. (2012). One ml of each milk sample was inoculated into 9 ml of sterile Peptone Water for enrichment and incubated overnight at 37°C. A loopful of broth culture was streaked on sterile MacConkey Agar (Oxoid, UK) and Eosin Methylene Blue (EMB) Agar (Oxoid, UK) plates using the quadrant streaking method and incubated aerobically at 37°C. The plates were checked for bacterial growth after 24, 48 and 72 hours to rule out slow growing bacteria. The colonies were examined for morphological features such as size, shape, and color. Pink colonies on MacConkey Agar, and greenish metallic sheen, purple, pink, blue-black, and orange colonies on EMB were sub cultured respectively on freshly prepared MacConkey Agar and EMB Agar plates and incubated at 37°C for 24 hours to get pure culture of coliform isolates. Representative colonies were stored on a slant of Nutrient Agar and kept in the refrigerator (4°C) until required for further work (David, 2011).

The presumptive Gram-stained coliforms were subjected to conventional biochemical tests namely, gelatin liquefaction, nitrate reduction, urease production, oxidase, Indole-methyl-red-Voges-Proskauer (IMVP), catalase, citrate agar, and sugar fermentation tests (Müller et al., 2003).

The confirmatory screening was carried out on presumptive Gram-stained coliforms using Oxiod™ Microbact™ GNB 24E according to the manufacturers' instructions. About 1 to 3 isolated colonies were picked from an 18 to 24 hours culture and emulsify in 5.0 ml of sterile saline and mixed thoroughly to obtain a homogeneous suspension. The plate containing the substrates was placed in the holding tray and using a sterile Pasteur pipette 4 drops (approximately 100 µl) of the bacterial suspension were added. Using a sterile pipette, the substrates underlined on the holding tray was overlayed with sterile mineral oil, in wells 1, 2, 3, 20 and 24. However, wells 8 and 20 were not overlayed with oil for oxidase- positive, miscellaneous Gram-negative bacilli. Incubation was done at 37°C for 18 to 24 hours and results were read as described by the manufacturer. The steps of the procedure were followed as prescribed by Balows et al. (1991).

Table 1. Primers used for amplification of ESBL genes.

Target	Primer name	Sequence (5' – 3')	Product size	References
Bla $_{TEM}$	TEM-F TEM-R	TCCGCTCATGAGACAATAACC TTGGTCTGACAGTTACCAATGC	931 bp	Kiratisin et al., 2008
Bla $_{CTX-M}$	CTX-F CTX-R	CGC TTT GCG ATG TGC AG ACC GCG ATA TCG TTG GT	550 bp	Villegas et al., 2004

Antimicrobial susceptibility testing

Antibiotic sensitivity testing was carried out on the coliforms isolated using the disc diffusion method the antibiotics employed were Ofloxacin (5 µg), Pefloxacin (5 µg), Erythromycin (15 µg), Ciprofloxacin (5 µg), Amixiclin/clavulanic acid (30 µg), Gentamycin (10 µg), Tetracycline (30 µg), Streptomycin (10 µg), Chloramphenicol (30 µg), and Ampicillin (10 µg). Colonies of the isolated coliforms were suspended into bottles of 9 ml sterile peptone water, standardized to 0.5 McFarland and incubated for 4 hours. A sterile swab stick was dipped into the standardized inoculum and excess fluid removed from the swab by pressing it on the side of the bottle. The swab was used to spread on the entire surface of dried Mueller Hinton agar plate and allowed to stand for 30 minutes. The antibiotic discs were placed on the plates aseptically on the surface of the seeded plates 15 mm apart. The plates were then incubated at 35 to 37°C for 18 to 24 hours. The diameter of the zone of inhibition around each was measured in millimetres (mm) using a plastic transparent ruler and compared against a reference standard which contains measurement ranges and their equivalent qualitative categories of susceptible/sensitive (CLSI, 2014).

Phenotypic assay for ESBL production

Following Ezeanya et al. (2017), the inocula of the isolated coliforms that have already been standardized to 0.5 McFarland standards were inoculated on Brilliance ESBL Chromogenic Culture Medium (Oxoid, UK). Inoculated plates were incubated at 37°C aerobically for 24 hours and 48 hours; change in color of colonies was observed and interpreted as per Oxoid, UK guidelines. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls.

PCR assays for TEM and CTX-M beta-lactamase genes

Conventional PCR was used for amplification and detection of *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$ genes as described previously (Chen et al., 2010). The reaction was performed in GeneAmp PCR system 9700 thermocycler (Applied Biosystems, USA) under the following conditions: Initial denaturation at 95°C for 4 minutes. 35 cycles of denaturation were conducted at 95°C for 1 minute. Annealing was done for 1 minute and at 48°C for *bla*$_{TEM}$ and 60°C for *bla*$_{SHV}$ and *bla*$_{CTX-M}$. Primer extension was at 72°C for 1 minute and the final extension step was extended to 5 minutes at 72°C for all genes. Amplified genes were separated by gel electrophoresis, in 1% (W/V) agarose gel submerged in TBE 0.5X (Tris/borate/EDTA) buffer. *Klebsiella pneumoniae* ATCC 700603 containing *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{TEM}$ genes was used as positive control while, *Escherichia coli* ATCC 25922 not containing the *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{TEM}$ genes were used as negative control. PCR products were detected with ethidium bromide fluorescence using the Bio-imaging system (VWR – Syngene, US). The specific primers used are shown in Table 1.

Amplicon sequencing and BLAST

Sequencing reactions were performed in the Master Cycler pro 384 (Eppendorf) using the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using the primer that was synthesized for the interested genes.

The fluorescent-labelled fragments were purified from the unincorporated terminators with the BigDye XTerminator® Purification Kit (Applied Biosystems). The samples were injected to electrophoresis in an ABI 3730xl DNA Analyzer (Applied Biosystems).

The sequences obtained from the samples were aligned with GenBank sequences using the Basic Local Alignment Search Tool (BLAST) and FASTA programs of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi) used to search databases for similar nucleotide sequences and more than 96% similarity was considered (Altschul et al., 1990).

Phylogenetic analysis

Nucleotides obtained from 16sRNA sequences from ESBL type coliforms were analyzed for diversity with highly similar sequences from different countries, having the highest percentage coverage query and identity from

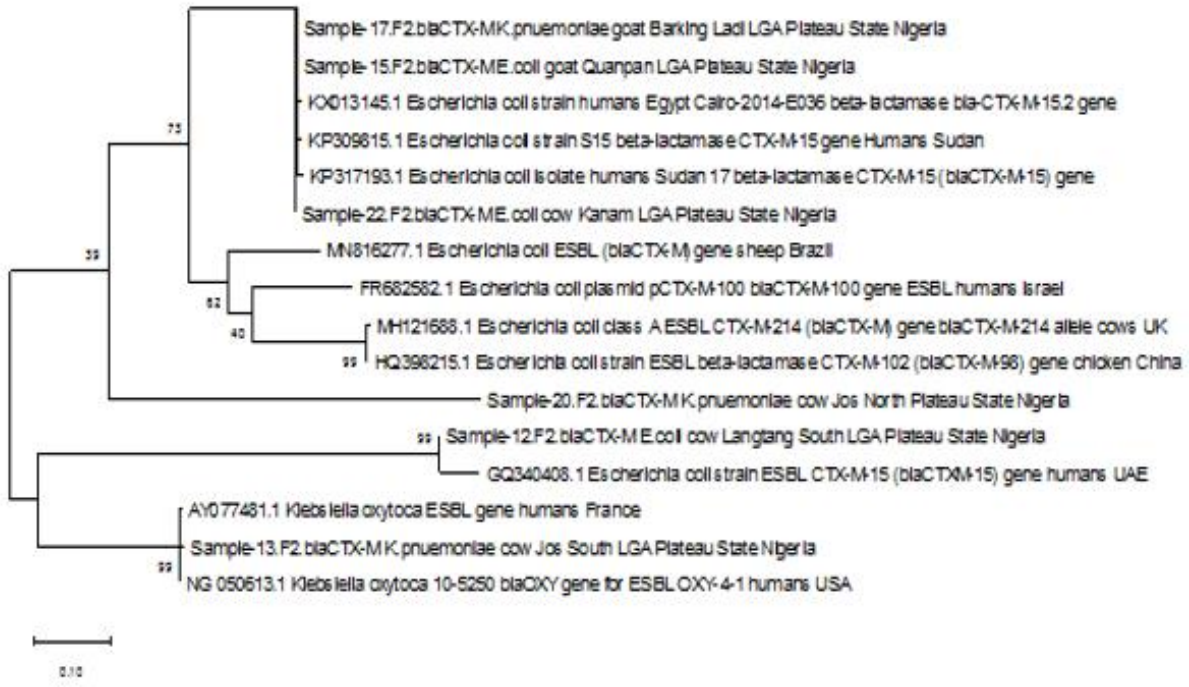

Figure 1. Phylodiversity of ESBL-types carrying bla_{CTX-M} gene recovered from ruminants from Plateau State Nigeria aligned and analysed with isolate sequences deposited at the GenBank using Maximum- likelihood algorithm in MEGA 10.0 with 500 bootstrap replicates.

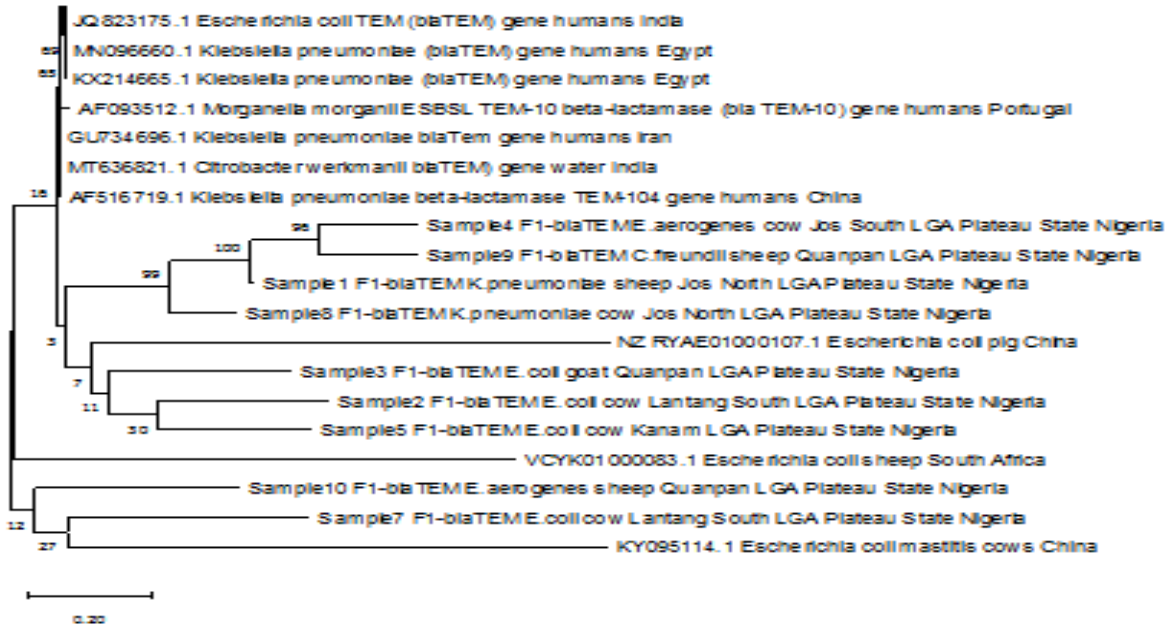

Figure 2. Phylodiversity of ESBL-types carrying bla_{TEM} gene recovered from ruminants from Plateau State Nigeria aligned and analysed with isolate sequences deposited at the GenBank using Maximum- likelihood algorithm in MEGA 10.0 with 500 bootstrap replicates.

GenBank database with their accession numbers as indicated in Figures 1 and 2. Nearest phylodiversity was determined by manually aligning the CTX-M and TEM individual gene sequences with homologous sequences configured into multiple sequence alignment using the Muscle program in MEGA software version 10.1.8

Table 2. Microbact identification of coliforms from ruminants.

Bacteria	Number (n = 677)	Percentage (%)
Escherichia coli	298	44.0
Klebsiella pneumonia	160	23.6
Klebsiella oxytoca	48	7.1
Citrobacter freundii	76	11.2
Enterobacter aerogene	68	10.1
Enterobacter cloacae	15	2.2
Serratia marcescens	12	1.8

$\chi^2 = 747.925$, $p < 0.000$ (statistically significant).

Table 3. Distribution of coliform isolates in each zone.

Zone	Number samples	Number of coliforms	Percentage (%)
Plateau North	251	183	72.9
Plateau Central	395	160	20.5
Plateau South	406	334	82.3
Total	1,052	677	64.4

$\chi^2 = 162.717$, $p < 0.000$ (statistically significant).

(http://www.megasoftware.net) and then construct tree by Maximum Likelihood method as independent, unordered and equally weighted according to Fitch parsimony with 500 heuristic bootstrap replicates and substitution model as “p” distance.

Data analysis

The Statistical Package for Social Science (SPSS) version 23 software was used to analyze data collected. Chi-square test (χ^2), and *P* values < 0.05 were considered statistically significant.

RESULTS

Isolation and identification

There were 677 coliforms isolated from 1052 milk samples across three zones of Plateau State of Nigeria where 183 (72.9%) were isolated from Plateau North, 160 (20.5%) from Plateau Central and 334 (82.3%) from Plateau South (Table 3). Table 2 shows that *Escherichia coli* (44.0%) was the most commonly isolated coliform, followed by *Klebiella pneumoniae* (23.6%), *Citrobacter freundii* (11.2%), *Enterobacter* aerogenes (10.1%), *K. oxytoca* (7.1%), *E. cloacae* (2.2%) and *Serratia marcescens* (1.8%). The isolation of coliforms was statistically significant (p<0.000) and their distribution in the three zones was also statistically significant (p<0.000) respectively.

Beta-lactamase production

Table 4 shows that out of the 677 coliforms isolated, 212 were ESBL producers. *E. Coli* had the highest prevalence of 48.58%, followed by *K. pneumoniae* (18.40%), *C. freundii* (12.74%), *E. aerogenes* (8.96%), *S. marcescens* (5.66%), *K. Oxytoca* (3.77%) and *E. cloacae* (1.89%). Plateau South had the highest number (132/62.3%) of ESB-producing coliforms while Plateau Central had the least (38/17.9%).

The antibiotic susceptibility testing of ESBL producing coliforms

When typed ESBL producing coliforms were subjected to the following commonly used antibiotics: Ofloxacin, Pefloxacine, , Ciprofloxacin, Amoxicillin-clavulanic acid, Gentamycin, and Streptomycin; *E. aerogenes* recorded the highest resistance (85.71%) against Gentamycin, followed by *Serratia marcescens* with an 81.82% resistance against Gentamycin, while the least resistant was *Serratia marcescens* against Pefloxacine, and followed by *K. oxytoca* and *E. cloacae* (25%) against Amoxicillin-clavulanic acid, and *E. cloacae* (25%) against Pefloxacine (Table 5).

Occurrence of ESBL variant genes in ruminant mastitis

Plates 1 to 8 showed that 36 ESBL producing genes from coliforms isolated from milk of cows, does and ewes with mastitis were identified by conventional PCR. The bands (560 bp) generated by PCR were identical to the bla_{CTX-M} gene of the control (Plates 1 to 6) while the bands (931 bp) were identical to the bla_{TEM} gene of the control (Plates 7 and 8). Table 6 summarizes Plates 1 to 8 which showed that blaCTX-M gene had a higher prevalence of 24.39% than blaTEM with 12.19%.

Table 4. Occurrence of ESBL-producing coliforms isolated from ruminant mastitis in Plateau State.

Zone	No of +ve samples (%)	Isolate (%)						
		E. Coli	*K. pneumoniae*	*K. Oxytoca*	*C. freundii*	*E. aerogenes*	*E. cloacae*	*S. marcescens*
P. North	42 (19.8)	21(50)	9 (21.43)	1(2.38)	4 (9.52)	6(14.28)	0(0.0)	1(2.38)
P. Central	38 (17.9)	28(73.68)	6(15.79)	0(0.0)	2(5.26)	2(5.26)	0(0.0)	0(0.0)
P. South	132 (62.3)	54(40.90)	24(18.18)	7(5.30)	21(15.90)	11(8.33)	4(3.03)	11(8.33)
Total	212	103(48.58)	39(18.40)	8(3.77)	27(12.74)	19(8.96)	4(1.89)	12(5.66)

Key: P. North – Plateau North; P. Central – Plateau Central; P. South – Plateau South.

Table 5. Percentage of resistant ESBL coliforms from ruminant mastitis to other antibiotics.

Isolate	No of isolate	OFX (%)	PEF (%)	CPX (%)	AMC (%)	CN (%)	S (%)
E coli	124	69 (55.64)	53(42.74)	46(37.09)	59(47.58)	89(71.77)	80(64.51)
K. pneumoniae	66	36 (54.54)	31(46.97)	33(50.00)	44(66.67)	34(51.51)	44(66.67)
K. oxytoca	8	5(62.50)	3(37.50)	4(50.00)	2(25.00)	5(62.50)	6(75.00)
C. freundii	45	28(62.22)	29(64.44)	22(48.89)	27(60.00)	33(73.33)	29(64.44)
E. aerogenes	14	11(78.57)	7(50.00)	7(50.00)	5(35.71)	12(85.71)	11(78.57)
E. cloacae	4	3(75.00)	1(25.00)	2(50.00)	1(25.00)	2(50.00)	3(75.00)
S. marcescens	11	3(27.27)	1(9.09)	3(27.27)	3(27.27)	9(81.82)	7(63.63)

Key: OFX: Ofloxacin; PEF: Pefloxacine; CPX: Ciprofloxacin; AMC: Amoxicillin-Clavulanic acid; CN: Gentamycin; S: Streptomycin.

Plate 1. Electrophoresis of PCR products for bla_{CTX-M}. Lane 2 – *K. pneumonia* from a cow, Lane 5 – *K. pneumonia* from a doe, Lane 6 – *K. pneumonia* from a cow, Lane 15 – *K. oxytoca* from a cow, Lane 19 – Control.

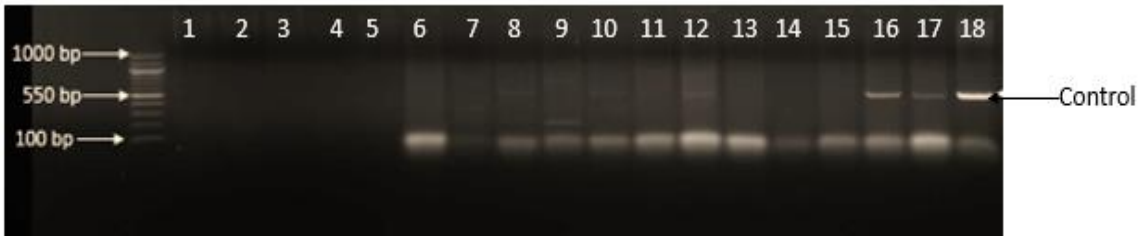

Plate 2. Electrophoresis of PCR products for bla_{CTX-M}. Lane 8 – *K. pneumonia* from a cow, Lane 10 – *E. coli* from a cow, Lane 12 – *K. pneumonia* from an ewe, Lane 16 – *C. freundii* from a cow, Lane 17 – *S. marcescens* from a cow, Lane 18 – Control.

Clonal diversity of CTX-M and TEM gene of isolated coliforms

The CTX-M and TEM gene sequences of the coliforms isolated from raw milk of cows with subclinical mastitis were aligned with sequences of published CTX-M and TEM genes respectively in coliform strains like *E. coli*, and *K. pneumoniae* strains from the GenBank (eg., *E. coli*

Plate 3. Electrophoresis of PCR products for bla$_{CTX-M}$. Lane 2 – *E. aerogenes* from an ewe**,** Lane 13 – *E. coli* from a doe**,** Lane 18 – *K.oxytoca* from a cow**,** Lane 19 – Control.

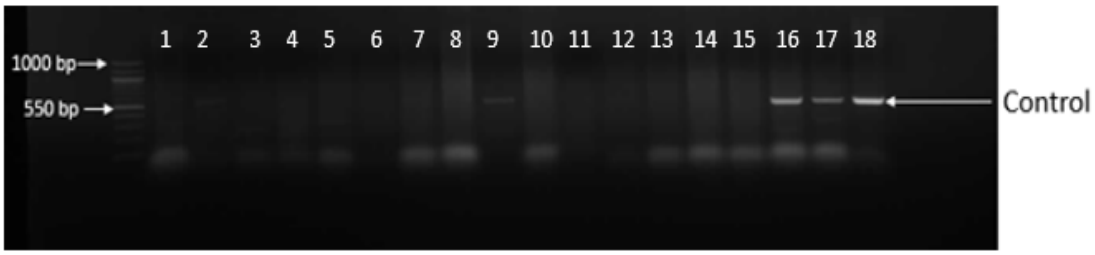

Plate 4. Electrophoresis of PCR products for bla$_{CTX-M}$. Lane 2 – *C. freundii* from a cow**,** Lane 9 – *E. coli* from an ewe**,** Lane 16 – *S. marcescens* from a cow**,** Lane 17 – *K. pneumonia* from a doe**,** Lane 18 – Control.

Plate 5. Electrophoresis of PCR products for bla$_{CTX-M}$. Lane 2 – *E. aerogenes* from an ewe**,** Lane 13 – *E. coli* from a doe**,** Lane 18 – *K.oxytoca* from a cow**,** Lane 19 – Control.

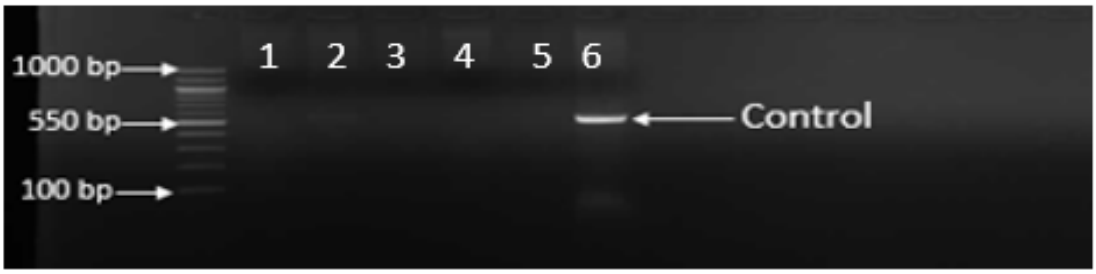

Plate 6. Electrophoresis of PCR products for bla$_{CTX-M}$. Lane 2 – *K. pneumonia* from a cow, Lane 6 – Control.

NZRYAE 1000107). The clonal diversity of the gene sequence of ESBL-types carrying bla$_{CTX-M}$ genes isolated in this study from Plateau State Nigeria showed clusters of 3 diverse clades (C) with other bacilli deposited in the GenBank (Figure 1). *K. pneumoniae* from cows in Jos South LGA of Plateau State Nigeria clustered with *K. oxytoca* from France and USA into clade 1 (C1). *E. coli* from cows in Langtang South LGA of Plateau State Nigeria

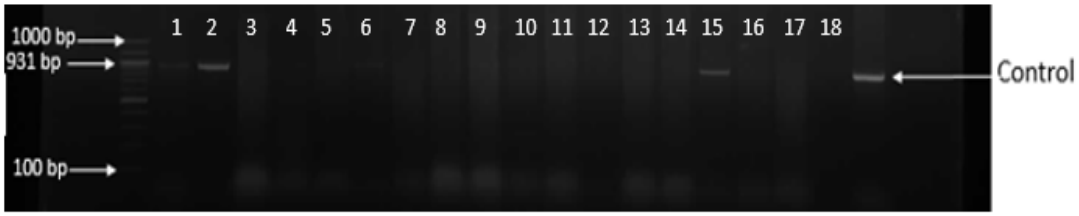

Plate 7. Electrophoresis of PCR products for bla_{TEM} . Lane 1 – *E. coli* from a doe**,** Lane 2 – *C. freundii* from a cow, Lane 6 – *E. coli* from an ewe, Lane 15 – *E. coli* from a cow, Lane 19 – Control.

Plate 8. Electrophoresis of PCR products for bla_{TEM}. Lane 1 – *E. coli* from a doe**,** Lane 2 – *C. freundii* from a cow, Lane 3 – *E. coli* from an ewe**,** Lane 4 – *E. coli* from a cow**,** Lane 5 – *K. pneumoniae* from a doe**,** Lane 10 – *E. aerogenes* from a cow**,** Lane 12 – *E. coli* from a doe**,** Lane 17 – *K.oxytoca* from a cow**,** Lane 19 – Control.

Table 6. Distribution of PCR-identified ESBL genes from ruminants with mastitis.

Coliform	No. sampled	No. ESBL genes (%)	
		bla_{TEM}	bla_{CTX-M}
E. coli	30	4 (13.33)	4 (13.33)
K. pneumoniae	22	1(4.55)	9 (40.90)
K. oxytoca	4	1(25.00)	2 (50.00)
E. aerogenes	14	2(14.28)	1 (7.14)
C. freundii	8	1 (12.50)	2 (25.00)
S. marcescens	4	1 (25.00)	2 (50.00)
Total	82	10 (12.19)	20 (24.39)

clustered with *E. coli* from humans in UAE into clade 2 (C2). *K. pneumoniae* from goats in Barkin Ladi LGA of Plateau State Nigeria clustered with *E. coli* from goats in Quanpan LGA of Plateau State Nigeria, *E. coli* from cows in Kanam LGA of Plateau State Nigeria, and *E. coli* from humans in Egypt and Sudan into clade 3 (C3).

The clonal diversity of the gene sequence of ESBL-types carrying bla_{TEM} genes isolated in this study from Plateau State Nigeria showed clusters of 2 diverse clades (C) with other bacilli deposited in the GenBank (Figure 2). *E. aerogenes* from sheep in Quanpan LGA of Plateau State Nigeria clustered with *E. coli* from a cow in Langtang South LGA of Nigeria and *E. coli* from a cow in China into (C4). High-level phylodiversity was shown for *E. aerogenes* from a cow in Jos South LGA of Plateau State Nigeria clustering with *C. freundii* in Quanpan LGA of Plateau State of Nigeria, *K. pneumoniae* from sheep in Jos North LGA of Plateau State Nigeria, *K. pneumoniae* from a cow in Jos North LGA of Plateau State Nigeria, *E. coli* from a pig in China, *E. coli* from goats in Quanpan LGA of Plateau State Nigeria, *E. coli* from a cow in Langtang LGA of Plateau State Nigeria and *E. coli* from a cow in Kanam LGA of Plateau State Nigeria into (C5).

DISCUSSION

Out of all the coliforms isolated from the ruminants, *E. coli* had the highest prevalence rate in all the cows sampled of 40.0%, followed by *K. pneumoniae* with a prevalence of 23.6% and the least was *S. marcescens* with prevalence of 1.8%. This is similar to a cross-sectional study carried

out in Gondar town, Ethiopia had 54 different bacterial species identified but, *E. coli* (29.6%), *Pseudomonas aeruginosa* (18.5%), and *Klebsiella pneumoniae* (16.7%), were the most commonly identified gram-negative staining bacterial pathogens (Garedew et al., 2012). Another study conducted in Sudan using raw milk, majority of the coliforms isolated were *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp. and *Citrobacter* with prevalence rates of 32, 29.2, 19.4, 11.1 and 1.0%, respectively (Salman and Hamad, 2011). This may be due to similar cultural livestock rearing practice where herds are moved from one location to another by pastoralists. Also, among the ewes, *E. coli* with 38% prevalence was the highest coliform isolated and the least was *S. marcescens* with 2.5%. Among the does, *E coli* had the highest prevalence as well with 43.1% and *E. aerogenes* the least with 1.5%. The isolation of coliforms in the current study was statistically significant ($p<0.000$). This meant that there is a likelihood that these organisms would occur and not due to chance. Plateau South Zone had the highest number (406) of coliforms isolated from ruminants while Plateau North had the least (251). The likelihood that isolates would occur in the distribution recorded in the three zones was also statistically significant ($p<0.000$).

From the coliforms isolated, ESBL producers were identified. European studies reported common occurrence of ESBL-producing *E. coli* in cattle in Switzerland (Geser et al., 2012), France (Madec et al., 2008), and the United Kingdom (Horton et al., 2011). Overall prevalence of ESBL producers in the ruminant mastitis was 31.3% with *E. coli* having the highest prevalence of 48.58%. This is in close agreement with the study carried out by Schmid et al. (2013) who also had a prevalence of 32.8% for ESBL-producing *E. coli.* Ali et al. (2016) had 23.53% prevalence of ESBL-producing *E. coli*. Klibi et al. (2019) reported that 10 out of 89 *E. coli* and 4 out of 30 *K. pneumoniae* isolated from milk with bovine mastitis were ESBL producers. Prevalence rate of ESBL-producing coliforms were found to be highest in Plateau South Zones with 62.3%. This could be because as heat and humidity increases, so does the bacterial multiplication as well as the load of pathogens in the environment. This is collaborated by a study done in India by Tiwari et al. (2013).

ESBL producers strains identified in this study carried either $bla_{CTX\text{-}M}$ or bla_{TEM}. Overall prevalence rate of $bla_{CTX\text{-}M}$ and bla_{TEM} in bovine mastitis was 24.39 and 12.19% respectively. Ali et al. (2016) reported that $bla_{CTX\text{-}M}$ was the predominant ESBL gene detected from bovine mastitis though with higher prevalence of 77.78% of the isolates. However, Yu et al. (2019) had a contrary result where bla_{TEM} was the most frequently detected resistance gene with 83.1% prevalence in bovine mastitis, followed by $bla_{CTX\text{-}M}$ with 66.3% prevalence. CTX-M has increasingly been identified in many different sources including humans, animals and the environment and that it has virtually displaced the other ESBLs within Entero-bacteriaceae during the last decade (Cantón et al., 2012).

Gentamycin showed the highest prevalence (85.17%) resistance to *E. aerogenes*, and *S. marcescens* (81.82%) while Amoxicillin-clavulanic acid and Pefloxacine showed the lowest resistance prevalence of 25% to *K. oxytoca* and *E. cloacae*. This agreed with Kibret and Abera (2011) where multiple antimicrobial resistances were 74.6% and increased resistance rates to all antimicrobials except ciprofloxacin. Fladberg et al. (2017) stated a lower prevalence of resistance (41%) to Gentamycin by *E. coli*.

Some of the clades show that CTX-M and the TEM genes from the ruminants have common ancestry with genes from human from different parts of the world. The high-level of phylodiversity observed among ruminants means that there is transfer coliform harboring the CTX-M and TEM genes which might have been transmitted from clones of varying origins (Jena et al., 2017).

Conclusion

The detection level of ESBL-producing coliforms amongst the isolates studied was 62.3 %. Subsequent work carried out on ESBL-producing coliforms from ruminants suffering mastitis must take ampC *β*-lactamase detection as well as other types of β-lactamases that may be present into account. This is because antibiograms may be hard to interpret due to the production of these enzymes together by the coliforms. The relatedness of the genes among organisms in different animals from diverse geographical location demands a quick intervention that would reduce mortality and morbidity of animals and zoonotic transmissions. Sustained hygienic standards of livestock housing, milking procedures and processes, culling of chronically affected livestock, regular laboratory screening of pastoralists for infections can serve as good interventions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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